Analysis of Zolpidem in Human Plasma by High-Performance Liquid Chromatography with Fluorescence Detection: Application to Single-Dose Pharmacokinetic Studies

Anna Liza B. Durol and David J. Greenblatt*

Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine and Division of Clinical Pharmacology, New England Medical Center Hospital, Boston, Massachusetts

Abstract

Zolpidem, an imidazopyradine hypnotic agent, can be quantitated by high-performance liquid chromatography (HPLC) with fluorescence detection. After the addition of a structurally related internal standard (propyl-zolpidem), plasma samples were double-extracted at neutral pH with toluene-isoamyl alcohol or benzene-isoamyl alcohol. The organic extracts were evaporated to dryness, reconstituted with mobile phase, and analyzed by HPLC using a C-18 reversed-phase column, a mobile phase of acetonitrile-50mM potassium dihydrogen phosphate (50:50), and fluorescence detection with excitation and emission wavelengths of 254 and 390 nm, respectively. The lower limit of reliable quantitation was in the range of 1-2.5 ng/mL. The method is applicable to single-dose pharmacokinetic studies of zolpidem in humans.

Introduction

Zolpidem (Figure 1) is an imidazopyridine derivative extensively prescribed as a hypnotic agent in clinical practice (1,2). Although not a benzodiazepine in structure, zolpidem acts via interaction with the central gamma-amino-butyric acid (GABA)-benzodiazepine receptor complex (1-5) and produces sedative, hypnotic, and amnestic effects similar to those associated with typical benzodiazepine receptor ligands (6-17). Because zolpidem has a very short elimination half-life (6), bedtime administration carries a low risk of residual daytime sedative effects (13-18).

A number of gas chromatographic and high-performance liquid chromatographic (HPLC) methods have been described for quantitation of zolpidem in plasma (19-26). The method described herein is rapid and sensitive and requires no special sample cleanup or derivatization. One individual can extract and prepare up to 96 samples in a single working day; analysis of the samples can be completed overnight using an automatic sampler.

Experimental

Instrumentation and reagents

A Waters Associates (Milford, MA) HPLC system consisted of a M-6000A solvent delivery system, M-730 data module, and a M-710B WISP automatic sampler. The column was a 30-cm x 3.9-mm reversed-phase C-18 μBondapak column (Waters). The mobile phase was HPLC-grade acetonitrile-50 mM potassium dihydrogen phosphate (50:50) at a flow rate of 1.5 mL/min. Column effluent was monitored with a Perkin-Elmer 650-10S fluorescence spectrophotometer operating at excitation and emission wavelengths of 254 and 390 nm, respectively. Analyses were performed at room temperature.

Toluene, benzene, isoamyl alcohol, and methanol were used as received from commercial sources. Pure zolpidem was purchased from Research Biochemical (Natick, MA) or provided by Lorex (Skokie, IL). Propyl-zolpidem, the internal standard (Figure 1), was provided by Synthelabo Research (Paris, France). Stock
Table I. Assay Variability Within and Between Days

<table>
<thead>
<tr>
<th>Added zolpidem concentration (ng/mL)</th>
<th>Measured concentration between days (ng/mL) mean (n = 8) %CV</th>
<th>Within-day % CV (n = 5–6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.98</td>
<td>12.8</td>
</tr>
<tr>
<td>2.5</td>
<td>2.42</td>
<td>5.2</td>
</tr>
<tr>
<td>5.0</td>
<td>5.00</td>
<td>1.7</td>
</tr>
<tr>
<td>7.5</td>
<td>7.51</td>
<td>1.8</td>
</tr>
<tr>
<td>10.0</td>
<td>9.97</td>
<td>4.7</td>
</tr>
<tr>
<td>15.0</td>
<td>14.5</td>
<td>5.8</td>
</tr>
<tr>
<td>20.0</td>
<td>19.4</td>
<td>4.2</td>
</tr>
<tr>
<td>25.0</td>
<td>24.1</td>
<td>1.4</td>
</tr>
<tr>
<td>35.0</td>
<td>34.3</td>
<td>1.5</td>
</tr>
<tr>
<td>50.0</td>
<td>49.7</td>
<td>2.1</td>
</tr>
<tr>
<td>75.0</td>
<td>75.7</td>
<td>1.1</td>
</tr>
<tr>
<td>100.0</td>
<td>100.2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

solutions of zolpidem and propyl-zolpidem were prepared by dissolving 10 mg of each in 100 mL of methanol. These were diluted 1:100 with methanol to yield 1-µg/mL working solutions. Another zolpidem working solution of 0.1 µg/mL was prepared by further dilution. All solutions were stored in the dark in glass-stoppered bottles at -20°C.

Sample preparation procedure
A 100-µL volume of propyl-zolpidem working solution (1 µg/mL), containing 100 ng propyl-zolpidem, was added to 13-mL round-bottom culture tubes for "unknown" samples and to a series of calibration tubes. Varying known amounts of zolpidem (0, 1, 2.5, 5, 7.5, 10, 15, 20, 25, 35, 50, 75, and 100 ng) were added to calibration tubes. The organic solvents were evaporated to dryness at 40–47°C under mildly reduced pressure in a vacuum oven.

Preliminary studies indicated that recovery and replicability were optimized using a double extraction procedure with either toluene or benzene as the organic solvent. Drug-free "control" bovine serum or human plasma (0.5–1.0 mL) was added to the calibration tubes, and 0.5–1.0 mL of "unknown" plasma added to all other tubes. Two milliliters of the extraction solvent (either toluene–isoamyl alcohol or benzene–isoamyl alcohol, 98.5:1.5) were added, and the samples were agitated on a vortex mixer for 60 s. After centrifugation for 10 min at 400 × g, the organic layer was transferred to a tapered glass tube. Two milliliters of the extraction solvent were added to the remaining aqueous phase, and the samples were reagitated on a vortex mixer and recentrifuged. The combined organic extracts were then evaporated to dryness at 40–47°C under mildly reduced pressure in a vacuum oven. The residue was redissolved in 150 µL of mobile phase and transferred to an HPLC autosampling vial with a limited volume insert; 10–20 µL was then injected onto the HPLC.

Single-dose pharmacokinetic study
Three healthy volunteer subjects participated after giving written informed consent. Each received a single 10-mg oral dose of zolpidem tartrate (Ambien, Searle, Chicago, IL), which is equivalent to 8.04 mg of zolpidem base, following an overnight fast. Venous blood samples were drawn into heparinized tubes...
before dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, and 24 h postdosing. Plasma was separated and frozen at -20°C until the time of assay. Concentrations of zolpidem were determined using the described procedure. Model-independent methods were used to determine the following pharmacokinetic variables: peak plasma concentration, time of peak concentration, apparent elimination half-life, total area under the plasma concentration curve, and oral clearance (27).

Results

Evaluation of the method

Under the described conditions, the retention times were 3.10 and 4.75 min for zolpidem and propyl-zolpidem, respectively (Figure 2). Drug-free plasma or serum samples after extraction contained no endogenous substances that interfered with peaks corresponding to zolpidem or the internal standard. The relation between zolpidem concentrations and peak-height ratio (versus internal standard) was linear (correlation coefficients always greater than 0.99), and the intercepts were not significantly different from zero (Figure 3). Based on the coefficient of variation (CV, standard deviation divided by mean, in percent), within- and between-day variabilities were 11 to 13% at 1.0 ng/mL, with CVs less than 10% at concentrations of 2.5 ng/mL and higher (Table I). Absolute recoveries of propyl-zolpidem and zolpidem were greater than 90%, and slopes of calibration curves for extracted samples were within 4% of calibration slopes for unextracted samples. Calibration slopes extracted with toluene and benzene differed by less than 5%. Calibration slopes of standard curves extracted from human plasma and bovine serum differed by less than 2%.

Extracted samples were stable for at least 20 h when left at room temperature or when subjected to two freeze-thaw cycles. Plasma samples containing zolpidem were stable for at least 20 h when left at room temperature.

The deviation between duplicate samples, which was calculated as the CV, was evaluated in a series of 180 plasma samples analyzed in the course of human pharmacokinetic studies. The overall mean CV was 2.0%, and CVs were less than 5% in 94% of samples. Figure 4 shows the distribution of CVs in relation to plasma zolpidem concentration, which indicated close replicability of duplicate samples even in the low range of plasma concentrations.

Pharmacokinetic results

Figure 5 shows plasma zolpidem concentrations from the pharmacokinetic study. Kinetic variables are shown in Table II.

Discussion

This report describes a reliable and specific method for the measurement of zolpidem in plasma using HPLC with fluorescence detection. The double-extraction procedure using either toluene–isoamyl alcohol or benzene–isoamyl alcohol yielded essentially complete recovery of zolpidem and the internal standard with no endogenous interfering peaks. Within- and between-day replicability data indicated a lower limit of reliable quantitation in the range of 1.0–2.5 ng/mL, which was more than sufficient for single-dose pharmacokinetic studies. The pharmacokinetic variables for zolpidem in the three subjects reported herein are highly consistent with previous kinetic studies of zolpidem (2,6,7,28).
Acknowledgment

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References


Table II. Kinetics of Zolpidem in Three Healthy Volunteers after a 10-mg Oral Dose of Zolpidem Tartrate

<table>
<thead>
<tr>
<th>Subject number</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23</td>
<td>32</td>
<td>25</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>78.2</td>
<td>84.1</td>
<td>74.5</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Peak plasma zolpidem concentration (ng/mL)</td>
<td>103</td>
<td>140</td>
<td>75</td>
</tr>
<tr>
<td>Time of peak (h)</td>
<td>1.0</td>
<td>0.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Elimination half-life (h)</td>
<td>2.2</td>
<td>2.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Total AUC* (ng/mL · h)</td>
<td>374</td>
<td>579</td>
<td>206</td>
</tr>
<tr>
<td>Oral clearance (mL/min)</td>
<td>358</td>
<td>231</td>
<td>651</td>
</tr>
</tbody>
</table>

* Total area under the plasma concentration curve.

Biotransformation of zolpidem in humans is mediated principally by Cytochrome P450-3A4, which is present in the liver and possibly in the gastrointestinal tract as well (29). The primary metabolites are formed by hydroxylation at different sites on the molecule, followed by further oxidation to form acid derivatives. The retention times of those metabolites using reversed-phase HPLC systems were considerably shorter than that of the parent compound (22,29). Therefore, even if the metabolic products appeared in plasma in significant amounts and were recovered by the extraction procedure, interference with analysis of the parent compound was very unlikely.


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